Sal I Bam H I

| |

---------- - pUC 19

|

Xba I

1 **ATGGAGTCAA AGTTTGCTCA CATCATTGTT TCTTTCTTC TTGCAACTTC**

original sequence - ag a

51 **CTTTGAAACT CTCTTGGCAC** GAAAAGAAAG Tgatggacca gagatcttag

mutagenic primer

101 aacTTCAAAA GGAATTTGAA TGCAATGGAA AACAAAGGTG GCCAGAACTT

151 ATTGGTGTAC CAACAAAGCT TGCTAAGGGG ATAATTGAGA AGGAAAATTC

201 ACTCATAACT AATGTTCAGA TACTACTGAA TGGTTCTCCA GTCACAATGG

251 ATTATCGTTG TAATCGAGTT CGTCTTTTTG ATAACATTTT GGGTGATGTT

301 GTACAAATTC CTAGGGTGGC TTAA

Figure 1

1 GAATTCCGCA AGGAgcacac ccgactatcc acctgCTGCA GAG**ATGGTGC**

upstream primer

51 **ACGCAACCTC CCCGCTGCTG CTGCTGCTGC TGCTCAGCCT GGCTCTGGTg**

cc t- original sequence

101 gctcccggga tccctaccag AAAGTGCTCG CTGACTGGGA AATGGACCAA

mutagenic primer

151 CGATCTGGGC TCCAACATGA CCATCGGGGC TGTGAACAGC AGAGGTGAAT

201 TCACAGGCAC CTACATCACA GCCGTAACAG CCACATCAAA TGAGATCAAA

251 GAGTCACCAC TGCATGGGAC ACAAAACACC ATCAACAAGA GGACCCAGCC

301 CACCTTTGGC TTCACCGTCA ATTGGAAGTT TTCAGAGTCC ACCACTGTCT

351 TCACGGGCCA GTGCTTCATA GACAGGAATG GGAAGGAGGT CCTGAAGACC

401 ATGTGGCTGC TGCGGTCAAG TGTTAATGAC ATTGGTGATG ACTGGAAAGC

451 TACCAGGGTC GGCATCAACA TCTTCACTCG CCTGCGCACA CAGAAGGAGT

501 GAGGATGGCC CCGCAAAGCC AGCAACAATG CCGGAGTGCT GACACTGCTT

Hind III

551 GTGATATTCC TCCCCAATAA AGCTTG

Figure 2

```
        EcoR I
          |
    1  GAATTCGCAT ATGGCTGAAG CTGGTATCAC CGGTACTTGG TACAACCAGC
   51  TGGGGTCTAC CTTCATCGTT ACCGCTGGTG CTGACGGTGC ACTGACCGGT
  101  ACTTACGAAA GCGCTGTTGG TAACGCTGAA AGCCGTTATG TTCTGACCGG
  151  TCGTTACGAC TCTGCTCCGG CTACCGACGG TTCTGGTACT GCTCTGGGTT
  201  GGACCGTTGC TTGGAAAAAC AACTACCGTA ACGCTCACTC TGCTACCACC
  251  TGGTCTGGCC AGTACGTTGG TGGTGCTGAA GCTCGTATCA ACACCCAGTG
  301  GCTGCTGACC TCTGGTACCA CCGAAGCTAA CGCTTGGAAA TCTACCCTGG
  351  TTGGTCACGA CACGTTCACC AAAGTTAAAC CGTCTGCTGC TTCTATCTAGA
                                                        |
                                                      Xba I
```

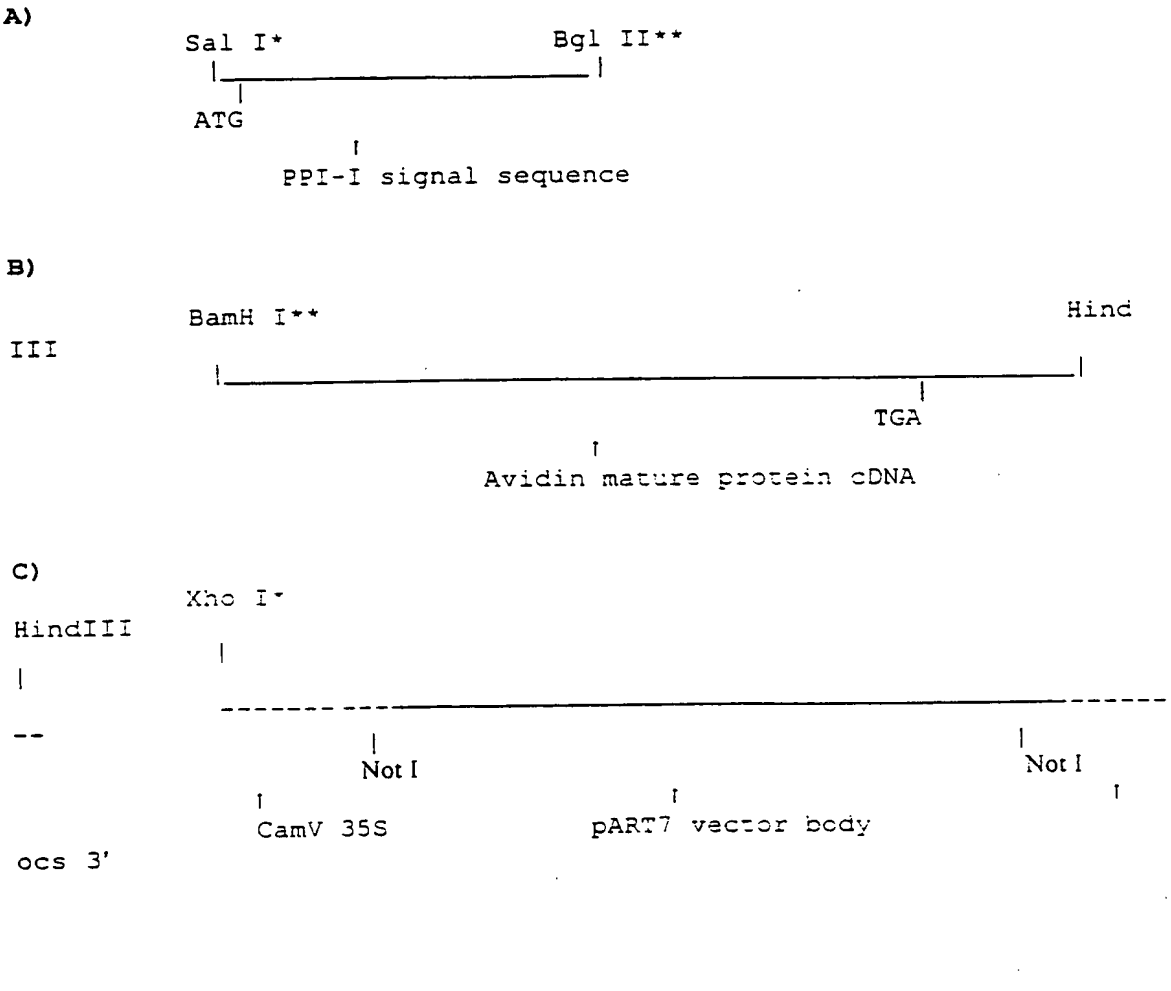
Figure 3

```
Sal I    altered Bam H I*
  |      |
---------- - pUC 19

     |
   Xba I
    1  ATGGATGTTC ACAAGGAAGT TAATTTCGTT GCTTACCTAC TAATTGTTCT
   51  TGGTAAGATT TTCCTTTACT CCTTTGTTTT AAAAAATAAA AAAACAAAAA
  101  AAATCTTGGT TTATACATAT ATATACACAC AAGTAGTTTT ATTTTTTTCC
  151  TTTATATTAT ATTTGTTGTA GGAATATTTC TACTTGTTAG CGTGGTGGAA
  201  CATGTTGATG CGAAGATCTG TACTAAAGAA TGTGGTAATC TTGGGTTTGG
  251  GATATGCCCA CGTTCAGAAG GAAGTCCGAA AAATCCCATA TGCATCAATT
  301  GTTGCTCAGG CTATAAGGGT TGTAATTATT ATAGTGTTTT CGGGAGATTT
  351  ATTTGCGAAG GAGAATCTGA CCTAAAAAAC CCAAAAGCTT GCCCCCTAAA
  401  TTGTGATACA AATATTGCCT ATTCAAGATG CCCCCATTCA GAAGGAAAAT
  451  CGCTAATTTA TCCCACCGGA TGTACCACAT GTTGCACAGG GTACAAGGGT
  501  TGCTACTATT TCGGTAAAAA TGGCAAGTTT GTATGCGAAG GAGAGAGTGA
  551  TGAACCCAAG GCAAATATGT ACCCTGCAAT GTGA
```

* result of PCR error during isolation of the PPI-II sequence

Figure 4



* compatible cohesive ends
** compatible cohesive ends

Figure 5

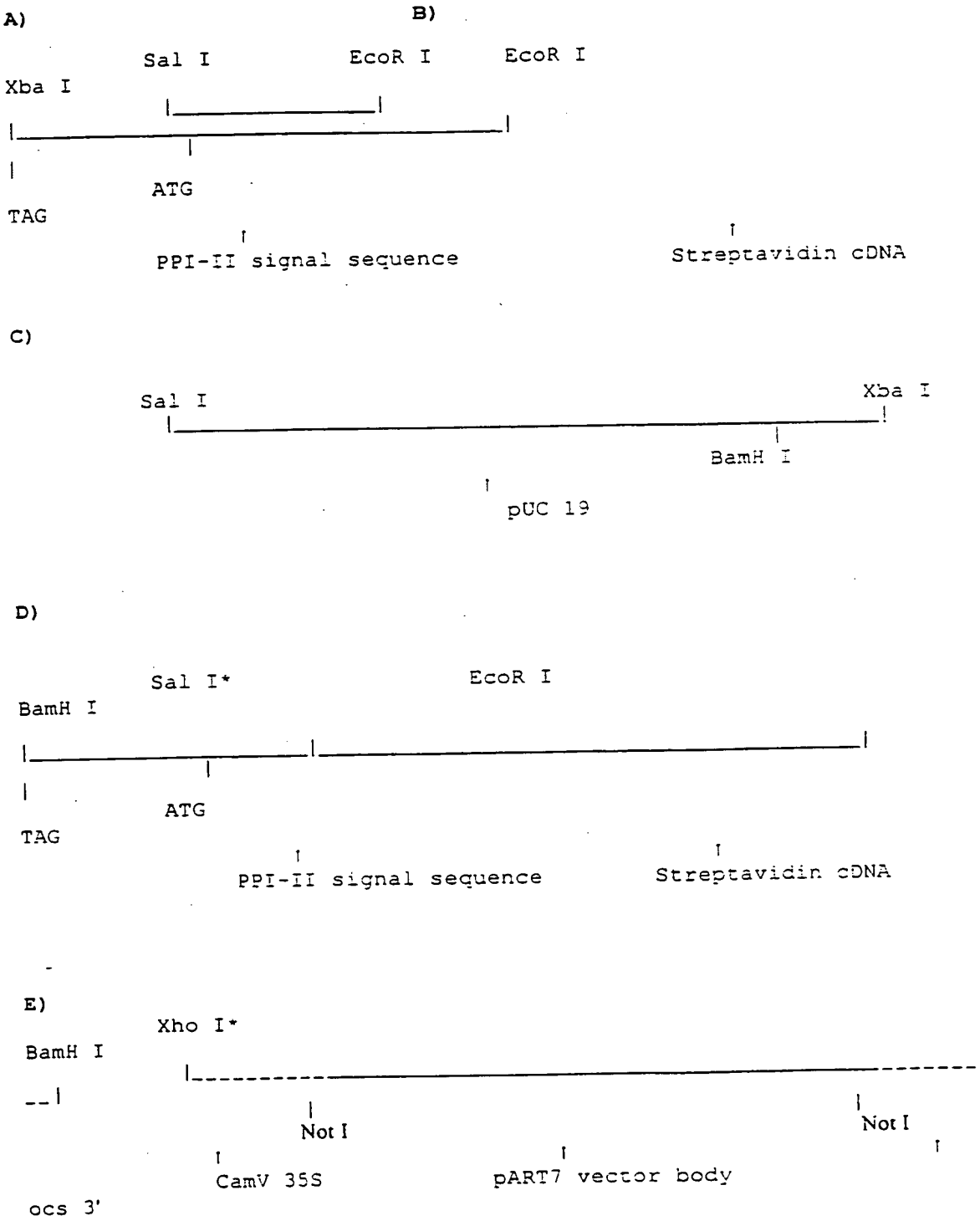
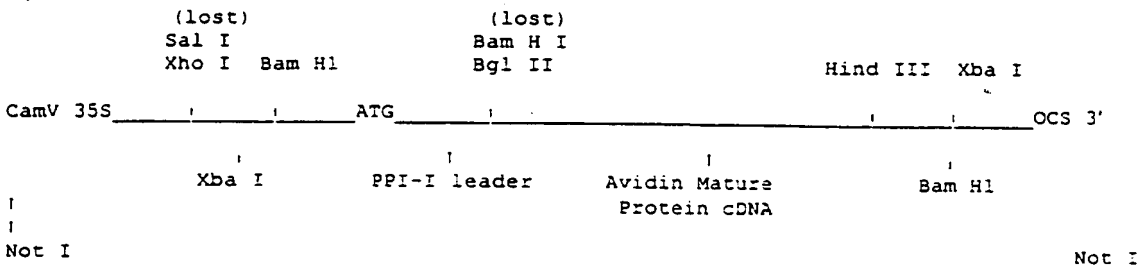

A)
B)
Sal I
EcoR I
EcoR I
Xba I
ATG
TAG
PPI-II signal sequence
Streptavidin cDNA
C)
Sal I
Xba I
BamH I
pUC 19
D)
Sal I*
EcoR I
BamH I
ATG
TAG
PPI-II signal sequence
Streptavidin cDNA
E)
Xho I*
BamH I
Not I
Not I
CamV 35S
pART7 vector body
ocs 3'
* compatible cohesive ends

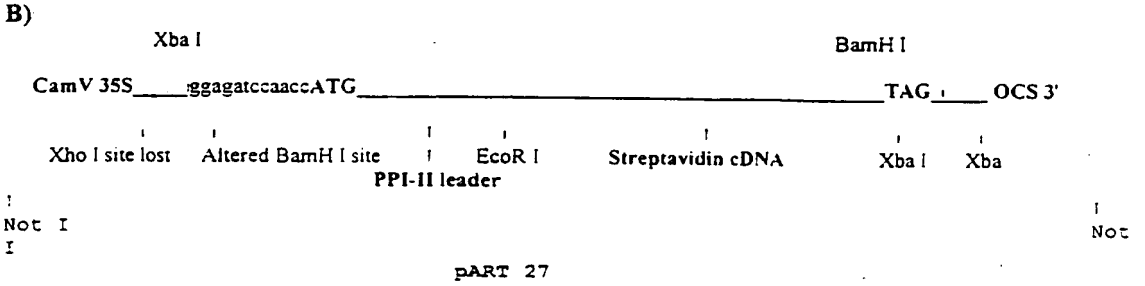
Figure 6

A)



pART27

B)



pART 27

Figure 7

A)

```
   1  ATGGAGTCAA AGTTTGCTCA CATCATTGTT TTCTTTCTTC TTGCAACTCC
  51  CTTTGAAACT CTCTTGGCAC GAAAAGAAAG TGATGGACCA GAGATCCCTG
 101  CCAGAAAGTG CTCGCTGACT GGGAAATGGA CCAACGATCT GGGCTCCAAC
 151  ATGACCATCG GGGCTGTGAA CAGCAGAGGT GAATTCACAG GCACCTACAT
 201  CACAGCCGTA ACAGCCACAT CAAATGAGAT CAAAGAGTCA CCATTGCATG
 251  GGACACAAAA CACCATCAAC AAGAGGACCC AGCCCACCTT TGGCTTCACC
 301  GTCAATTGGA AGTTTTCAGA GTCCACCACT GTCTTCACGG GCCAGTGCTT
 351  CATAGACAGG AATGGGAAGG AGGTCCTGAA GACCATGTGG CTGCTGCGGT
 401  CAAGTGTTAA TGACATTGGT GATGACTGGA AAGCTACCAG GGTCGGCATC
 451  AACATCTTCA CTCGCCTGCG CACACAGAAG GAGTGA
```

B)

cleavage site

```
   1  MESKFAHIIV FFLLATPFET LLARKESDGP EIpARKCSLT GKWTNDLGSN
  51  MTIGAVNSRG EFTGTYITAV TATSNEIKES PLHGTQNTIN KRTQPTFGFT
 101  VNWKFSESTT VFTGQCFIDR NGKEVLKTMW LLRSSVNDIG DDWKATRVGI
 151  NIFTRLRTQK E*
```

Figure 8

A)

```
   1  ATGGATGTTC ACAAGGAAGT TAATTTCGTT GCTTACCTAC TAATTGTTCT
  51  TGGTAAGATT TTCCTTTACT CCTTTGTTTT AAAAAATAAA AAAACAAAAA
 101  AAATCTTGGT TTATACATAT ATATACACAC AAGTAGTTTT ATTTTTTTCC
 151  TTTATATTAT ATTTGTTGTA GGAATATTTC TACTTGTTAG CGTGGTGGAA
 201  CATGTTGATG CGAAGATCTG TACTAAGAAT TCGCATATGG CTGAAGCTGG
 251  TATCACCGGT ACTTGGTACA ACCAGCTGGG GTCTACCTTC ATCGTTACCG
 301  CTGGTGCTGA CGGTGCACTG ACCGGTACTT ACGAAAGCGC TGTTGGTAAC
 351  GCTGAAAGCC GTTATGTTCT GACCGGTCGT TACGACTCTG CTCCGGCTAC
 401  CGACGGTTCT GGTACTGCTC TGGGTTGGAC CGTTGCTTGG AAAAACAACT
 451  ACCGTAACGC TCACTCTGCT ACCACCTGGT CTGGCCAGTA CGTTGGTGGT
 501  GCTGAAGCTC GTATCAACAC CCAGTGGCTG CTGACCTCTG GTACCACCGA
 551  AGCTAACGCT TGGAAATCTA CCCTGGTTGG TCACNACACG TTCACCAAAG
 601  TTAAACCGTC TGCTGCTTCT ATCTAG
```

B)

```
                                    cleavage site
                                          |
   1  MDVHKEVNFV AYLLIVLGIF LLVSVVEHVD AKICTKnshM AEAGITGTWY
  51  NQLGSTFIVT AGADGALTGT YESAVGNAES RYVLTGRYDS APATDGSGTA
 101  LGWTVAWKNN YRNAHSATTW SGQYVGGAEA RINTQWLLTS GTTEANAWKS
 151  TLVGHDTFTK VKPSAASI*
```

Figure 9

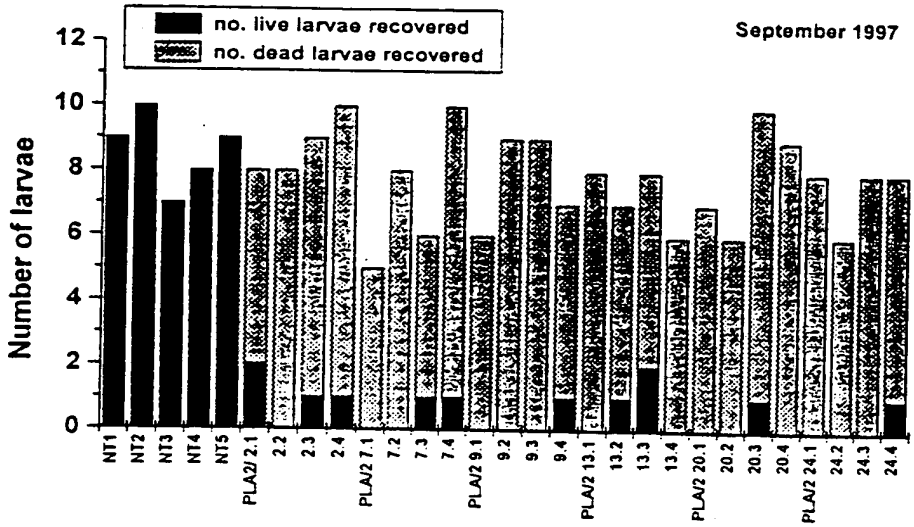


Figure 10

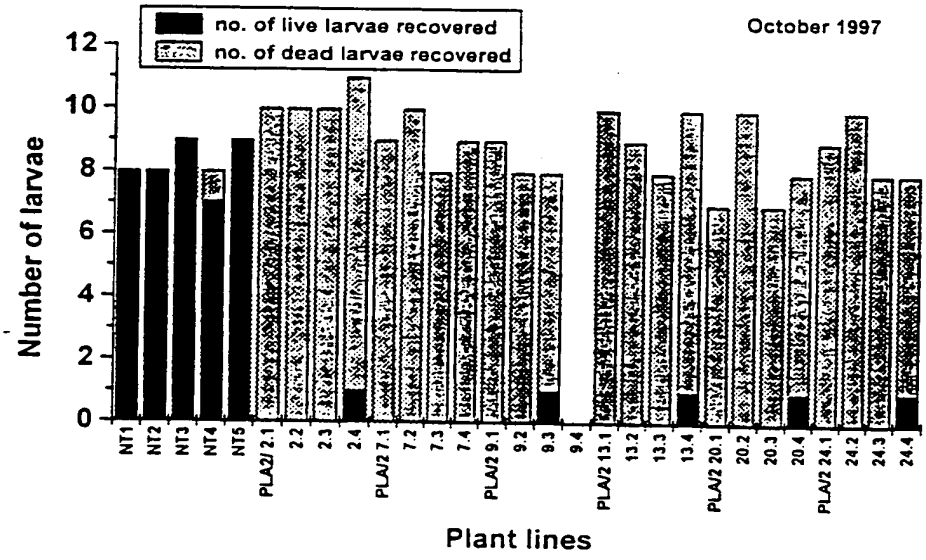


Figure 11

A)

```
   1  CCCTCCGTCC CCGCCGGGCA ACAACTAGGG AGTATTTTTC GTGTCTCACA
  51  TGCGCAAGAT CGTCGTTGCA GCCATCGCCG TTTCCCTGAC CACGGTCTCG
 101  ATTACGGCCA GCGCTTCGGC AGACCCCTCC AAGGACTCGA AGGCCCAGGT
 151  CTCGGCCGCC GAGGCCGGCA TCACCGGCAC CTGGTACAAC CAGCTCGGCT
 201  CGACCTTCAT CGTGACCGCG GGCGCCGACG GCGCCCTGAC CGGAACCTAC
 251  GAGTCGGCCG TCGGCAACGC CGAGAGCCGC TACGTCCTGA CCGGTCGTTA
 301  CGACAGCGCC CCGGCCACCG ACGGCAGCGG CACCGCCCTC GGTTGGACGG
 351  TGGCCTGGAA GAATAACTAC CGCAACGCCC ACTCCGCGAC CACGTGGAGC
 401  GGCCAGTACG TCGGCGGCGC CGAGGCGAGG ATCAACACCC AGTGGCTGCT
 451  GACCTCCGGC ACCACCGAGG CCAACGCCTG GAAGTCCACG CTGGTCGGCC
 501  ACGACACCTT CACCAAGGTG AAGCCGTCCG CCGCCTCCAT CGACGCGGCG
 551  AAGAAGGCCG GCGTCAACAA CGGCAACCCG CTCGACGCCG TTCAGCAGTA
 601  GTCGCGTCCC GGCACCGGCG GGTGCCGGGA CCTCGGCC
```

B)

```
   1  MRKIVVAAIA VSLTTVSITA SASADPSKDS KAQVSAAEAG ITGTWYNQLG
  51  STFIVTAGAD GALTGTYESA VGNAESRYVL TGRYDSAPAT DGSGTALGWT
 101  VAWKNNYRNA HSATTWSGQY VGGAEARINT QWLLTSGTTE ANAWKSTLVG
 151  HDTFTKVKPS AASIDAAKKA GVNNGNPLDA VQQ
```

Figure 12

Figure 13

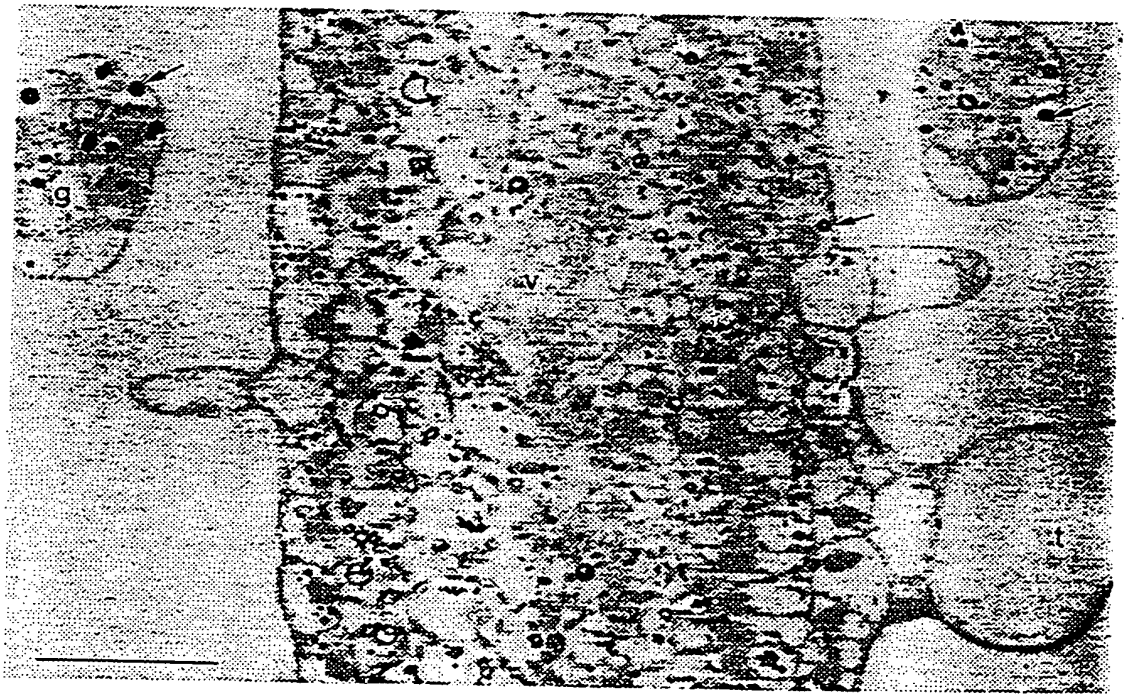


Figure 14

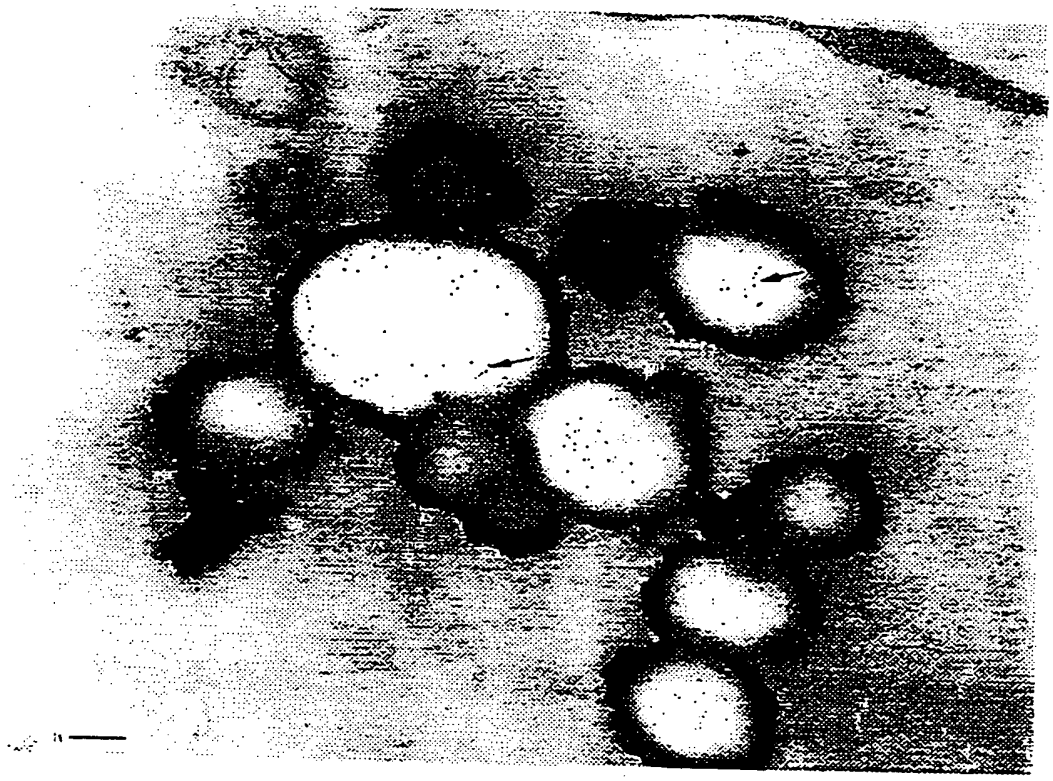


Figure 15

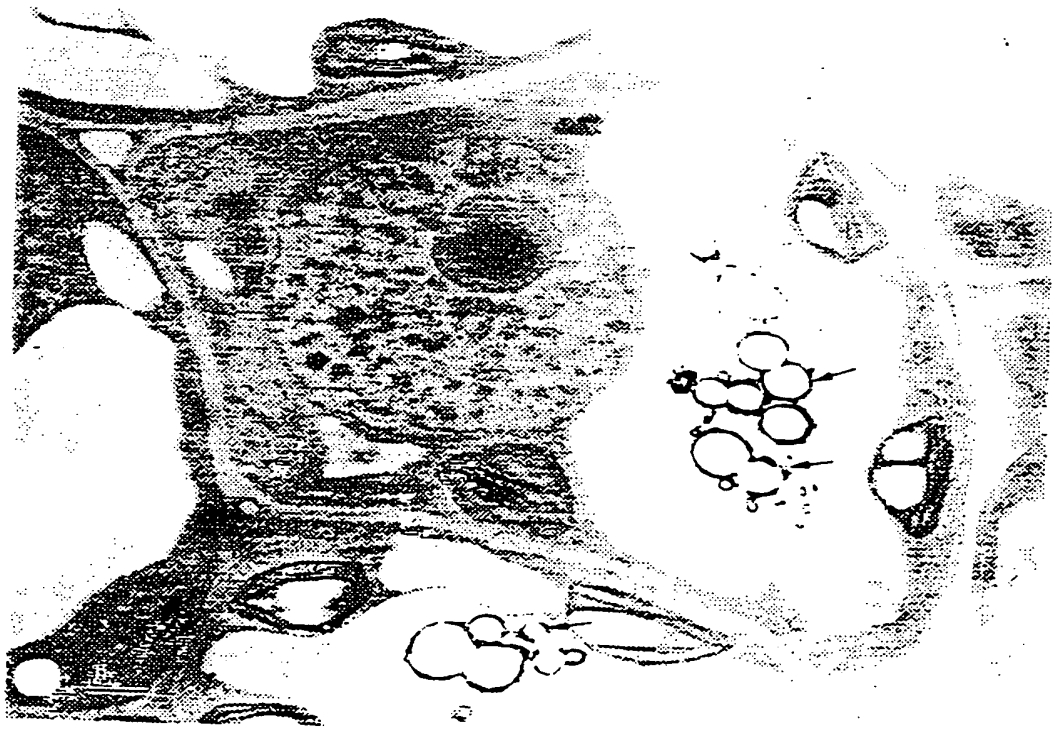


Figure 16

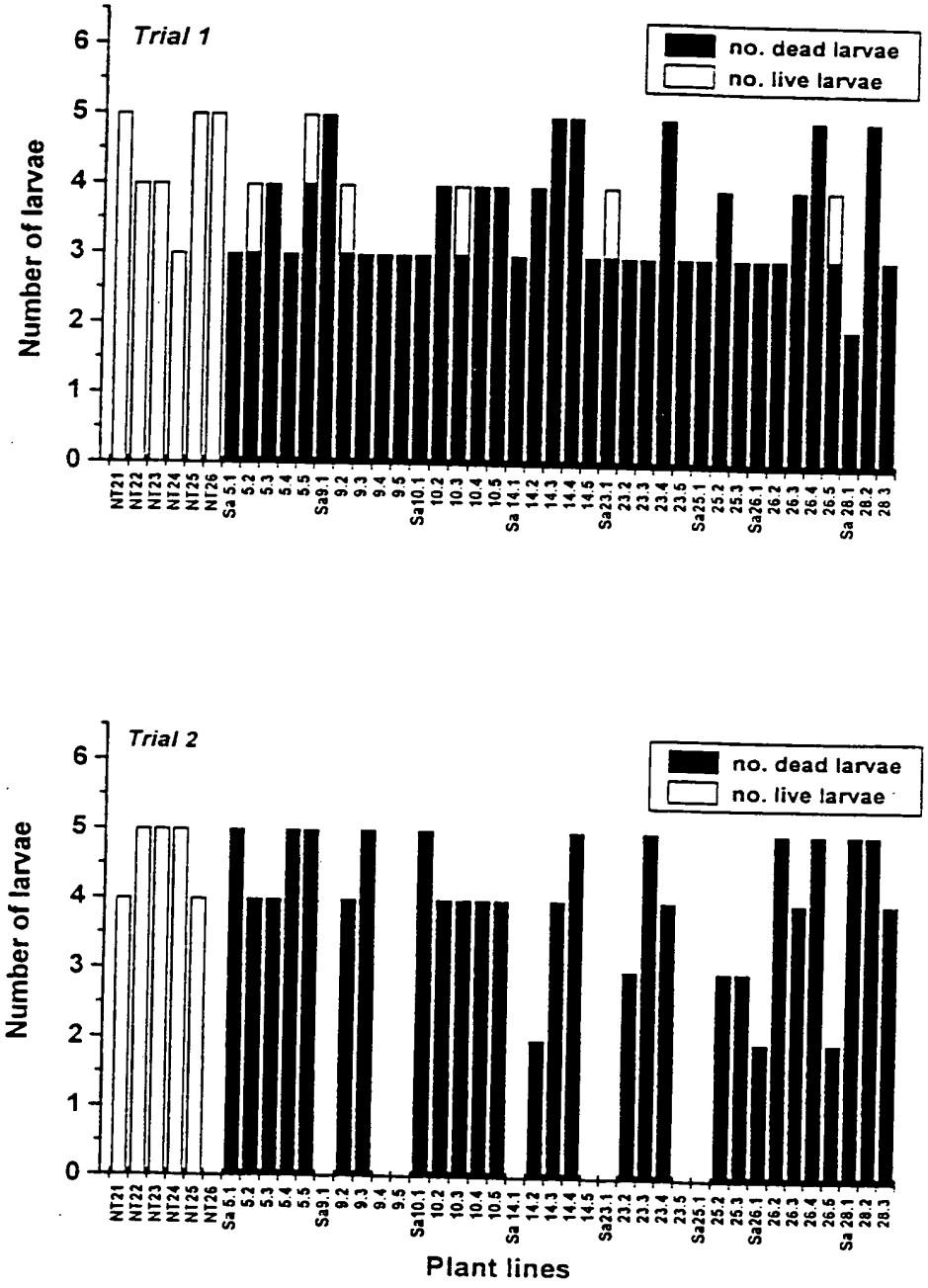

Trial 1
no. dead larvae
no. live larvae
Number of larvae
0
1
2
3
4
5
6
NT21
NT22
NT23
NT24
NT25
NT26
Sa 5.1
5.2
5.3
5.4
5.5
Sa9.1
9.2
9.3
9.4
9.5
Sa10.1
10.2
10.3
10.4
10.5
Sa 14.1
14.2
14.3
14.4
14.5
Sa23.1
23.2
23.3
23.4
23.5
Sa25.1
25.2
25.3
Sa26.1
26.2
26.3
26.4
26.5
Sa 28.1
28.2
28 3
Trial 2
no. dead larvae
no. live larvae
Number of larvae
Plant lines

Figure 17

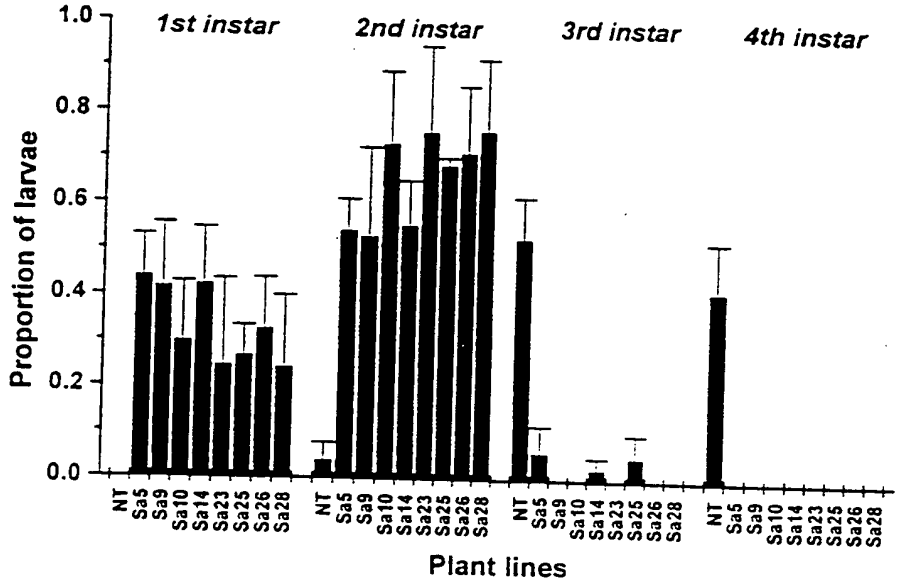


Figure 18

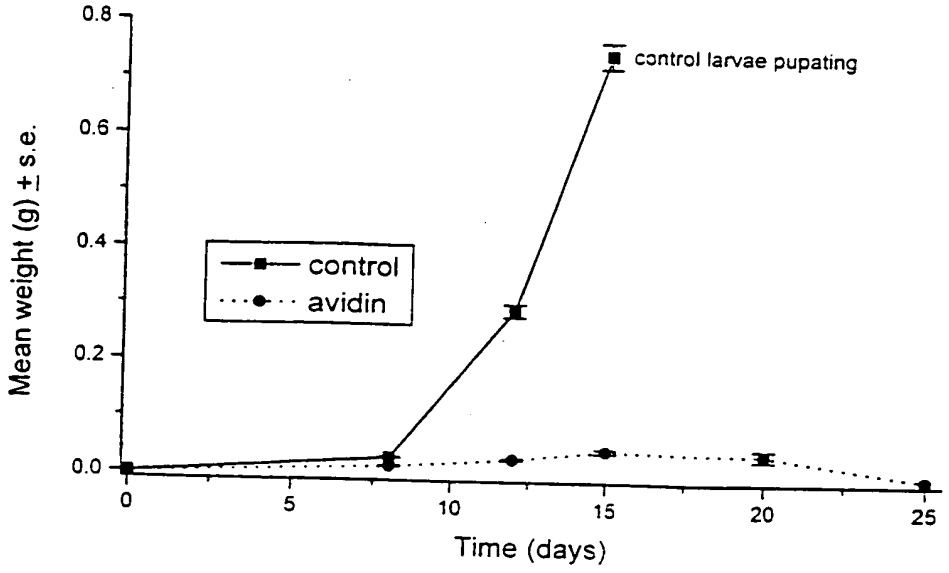


Figure 19A

Figure 19B

Figure 19C

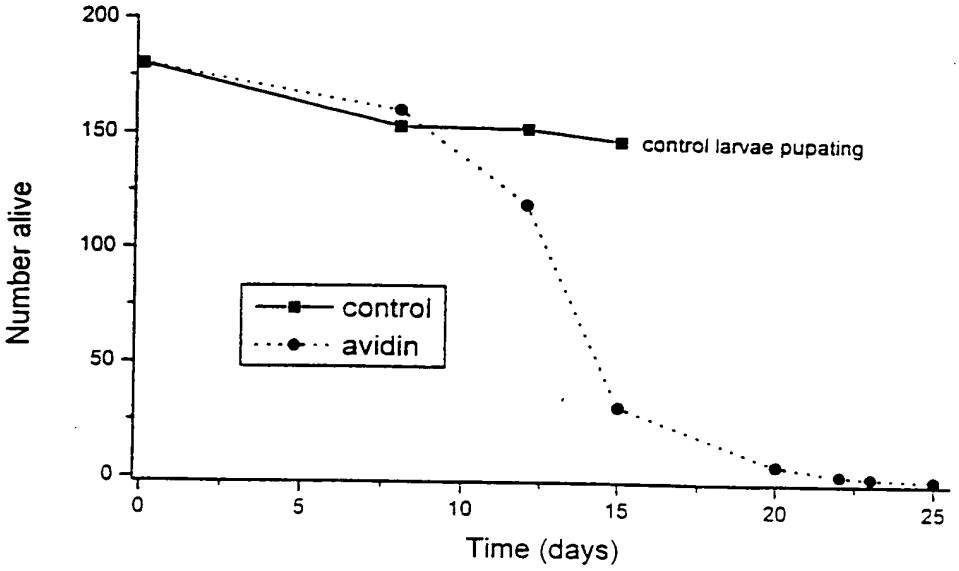


Figure 20

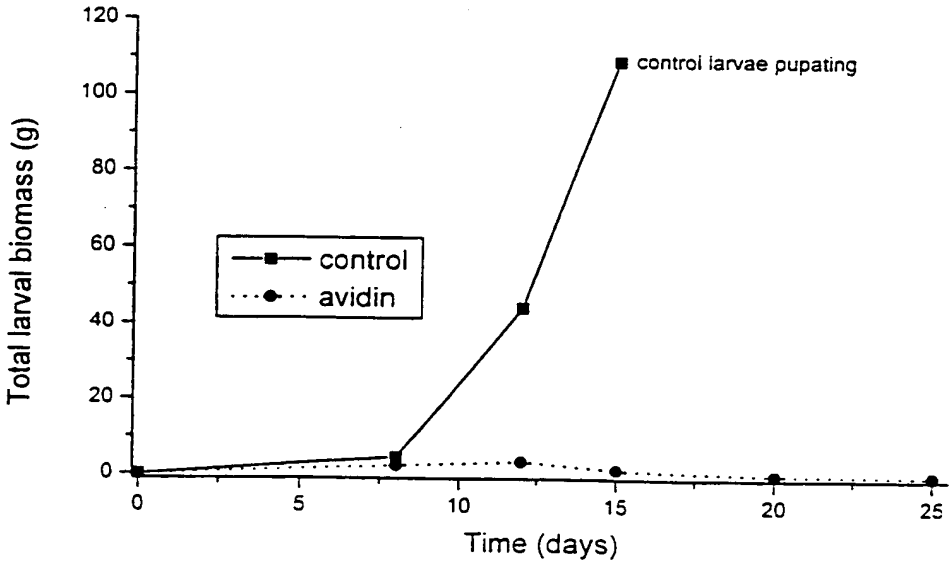


Figure 21

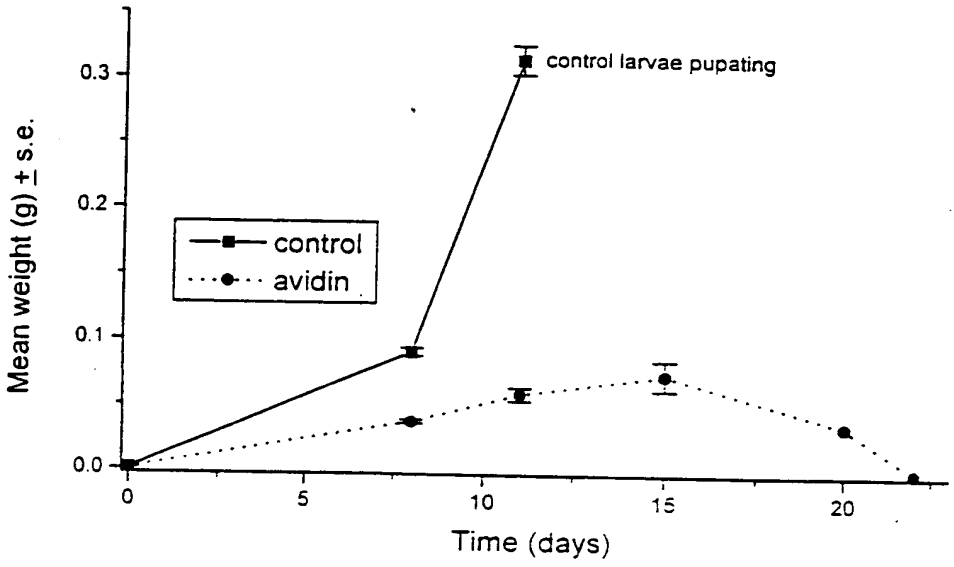

Mean weight (g) ± s.e.
0.3
0.2
0.1
0.0
control
avidin
control larvae pupating
0
5
10
15
20
Time (days)

Figure 22A

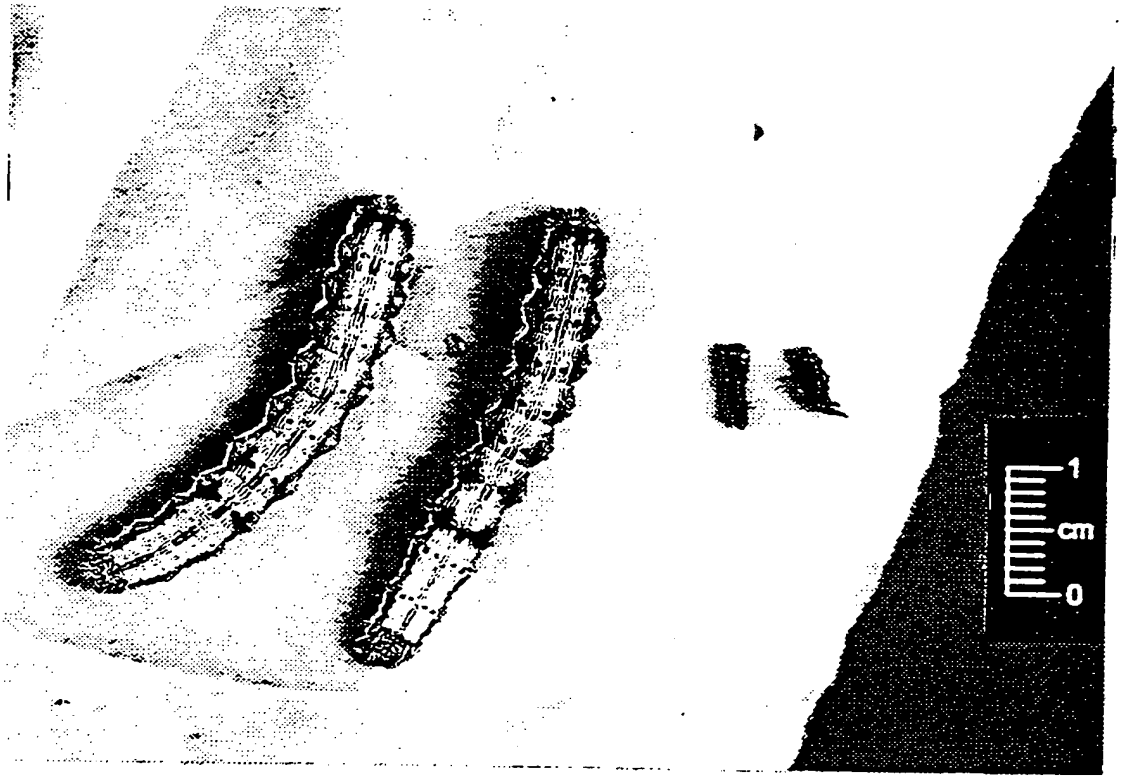


Figure 22B

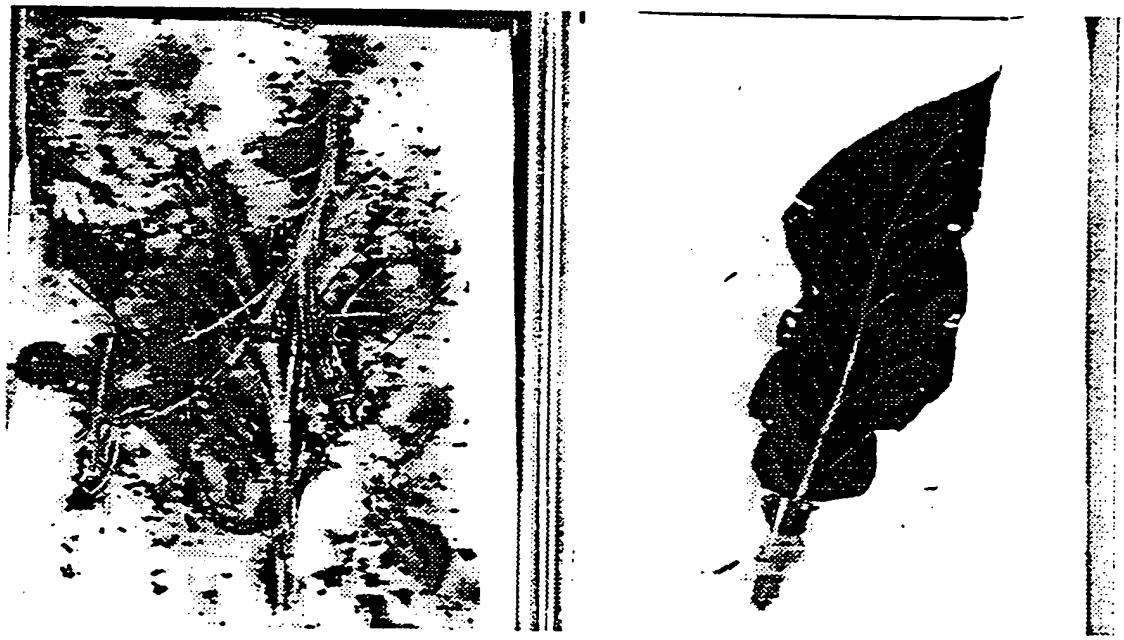


Figure 22C

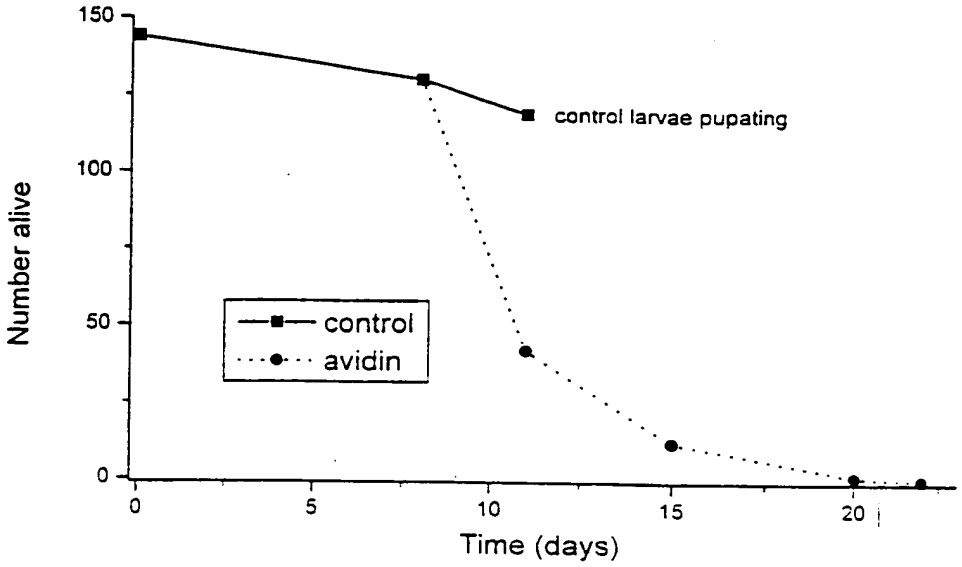

150
100
50
0
Number alive
0
5
10
15
20
Time (days)
control
avidin
control larvae pupating

Figure 23

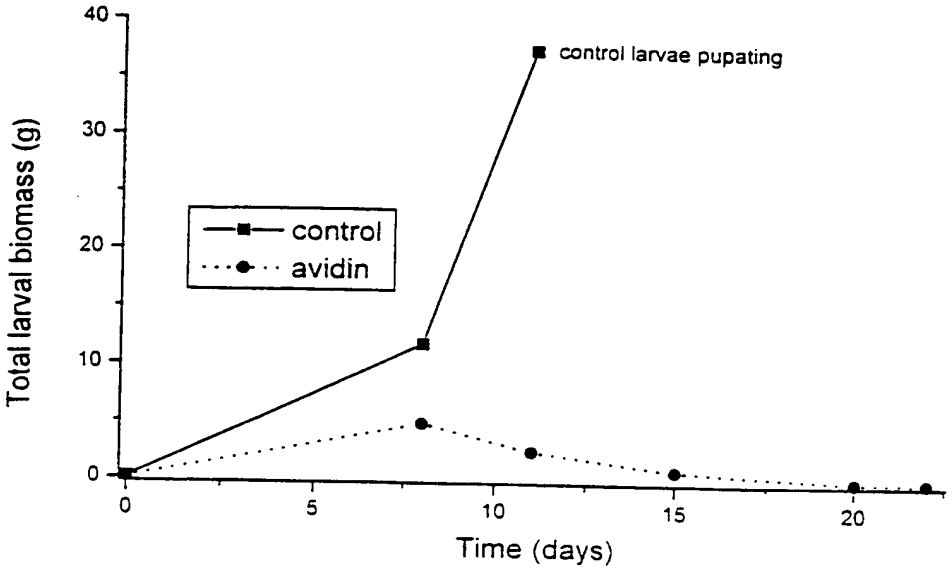


Figure 24

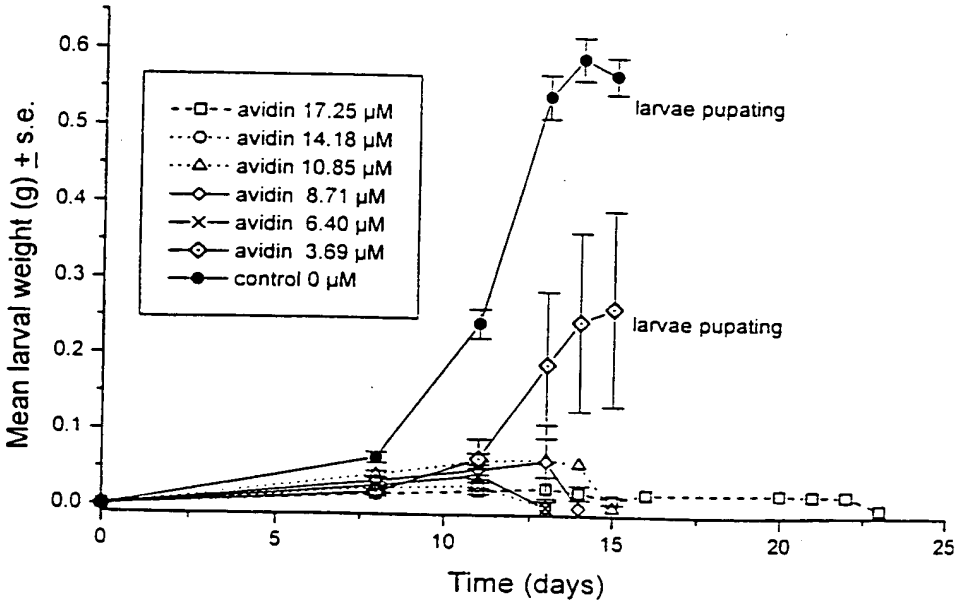


Figure 25

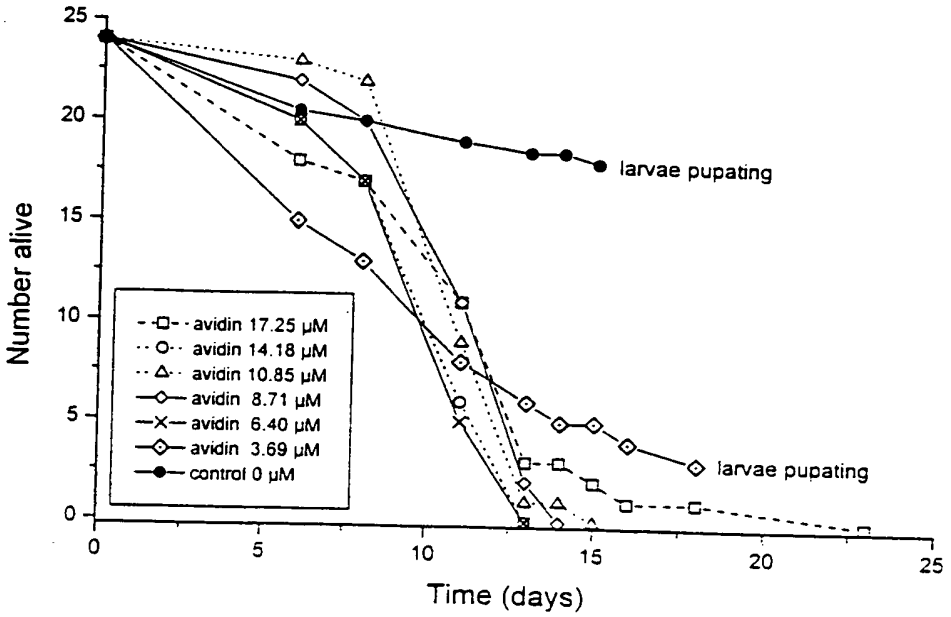


Figure 26

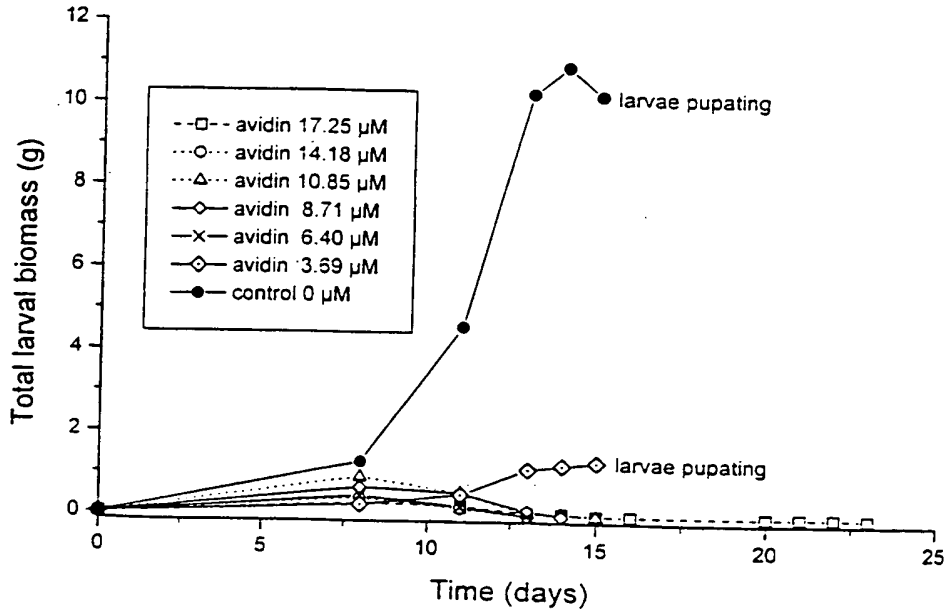


Figure 27

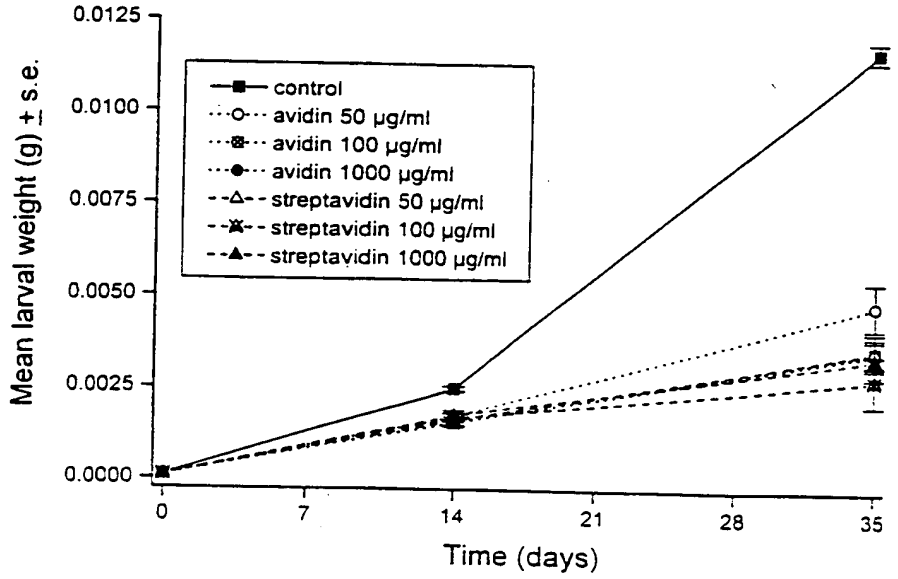


Figure 28

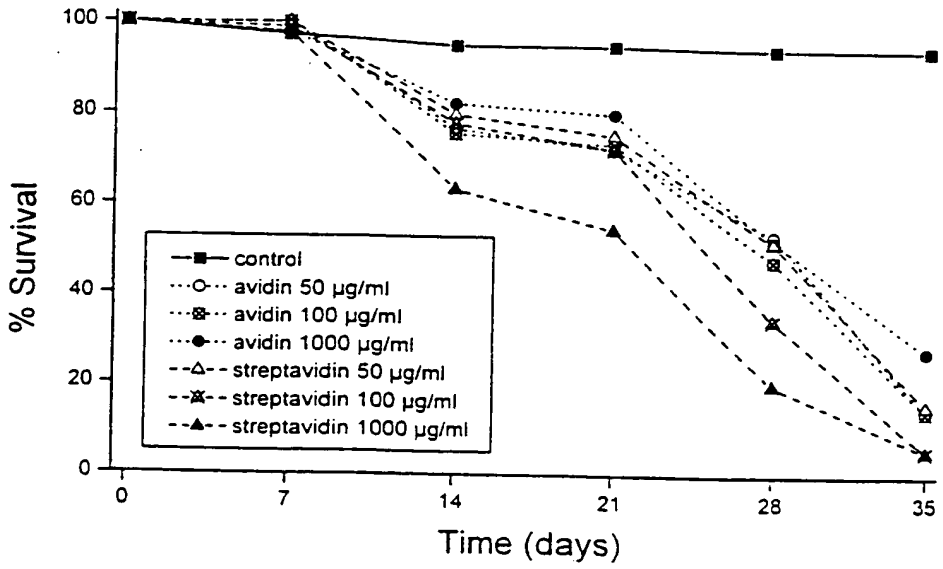


Figure 29

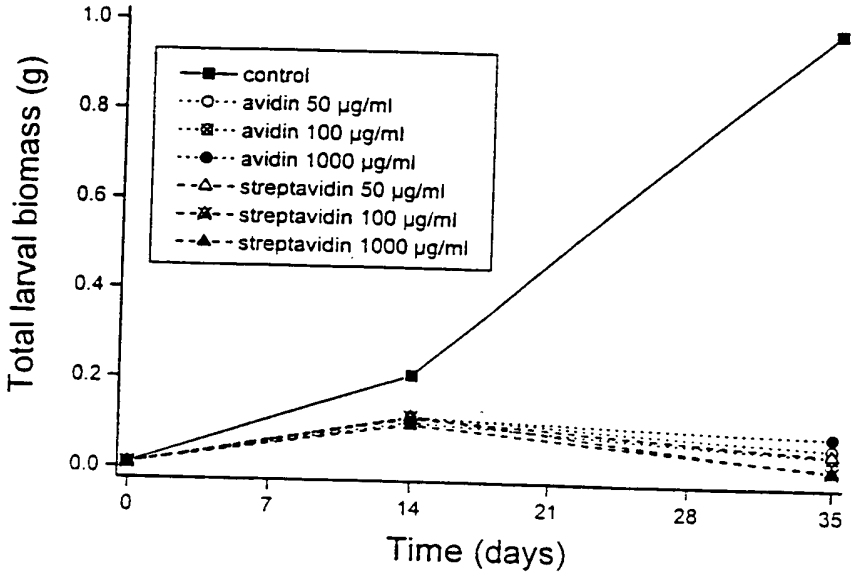


Figure 30

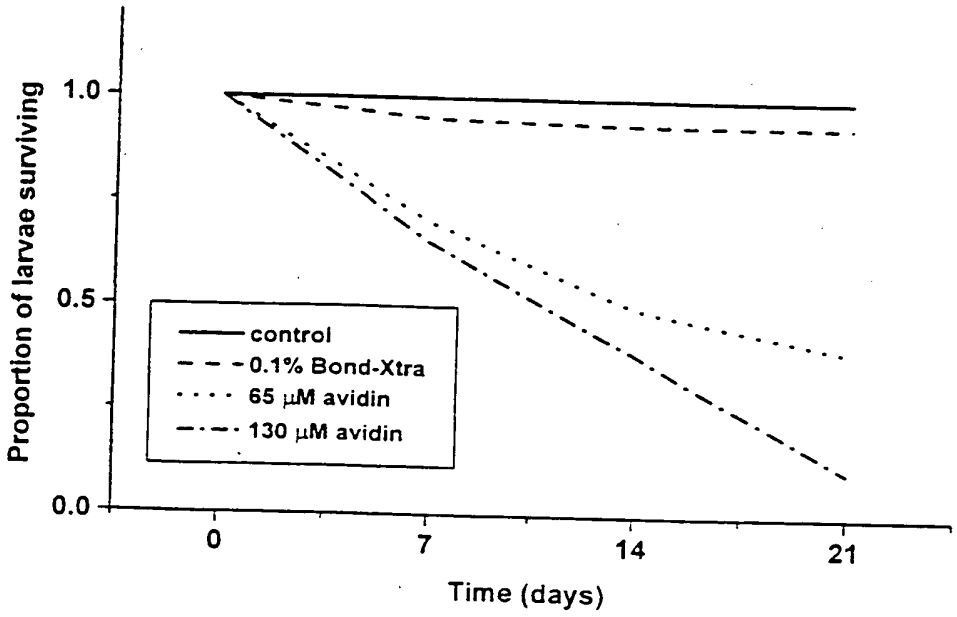


Figure 31

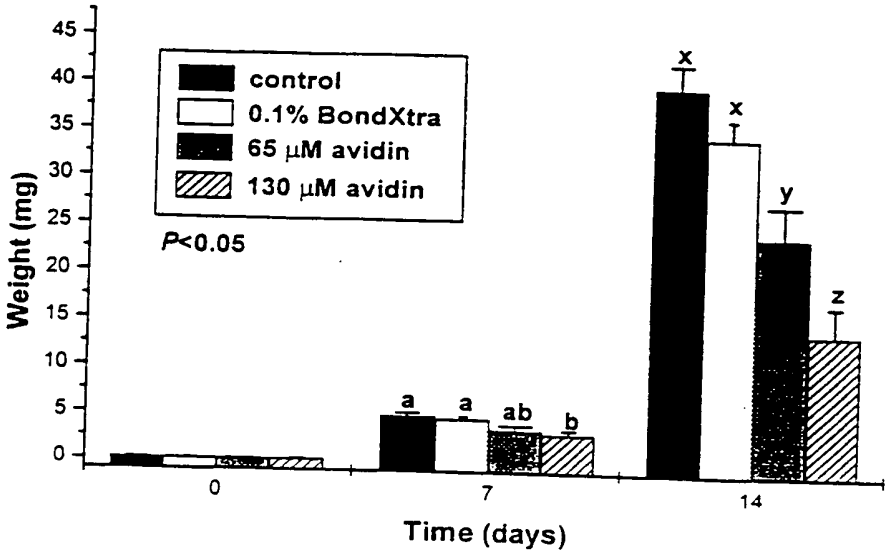


Figure 32

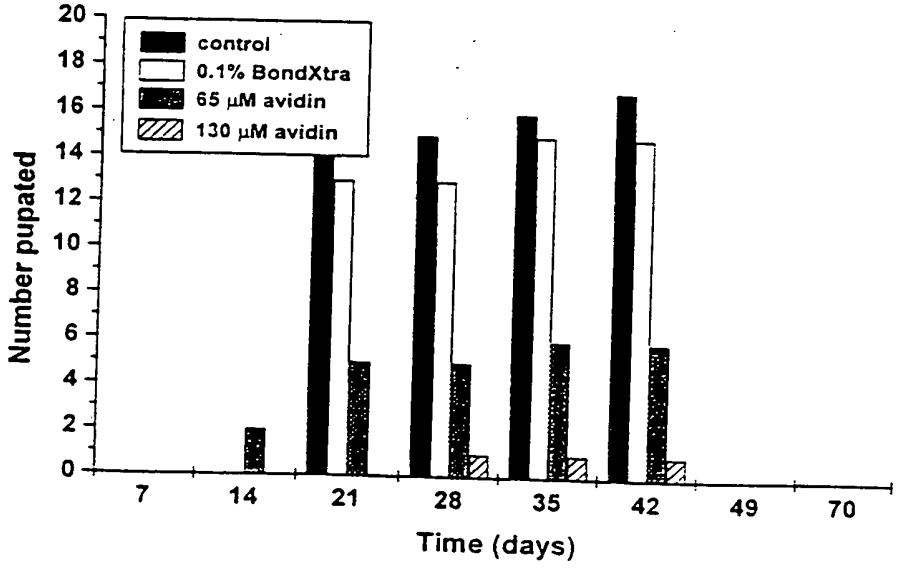


Figure 33

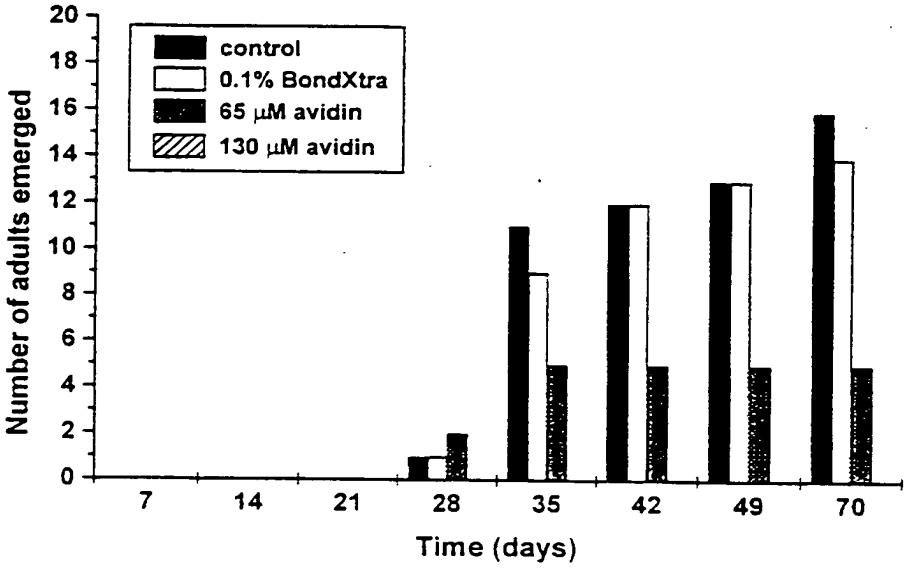


Figure 34

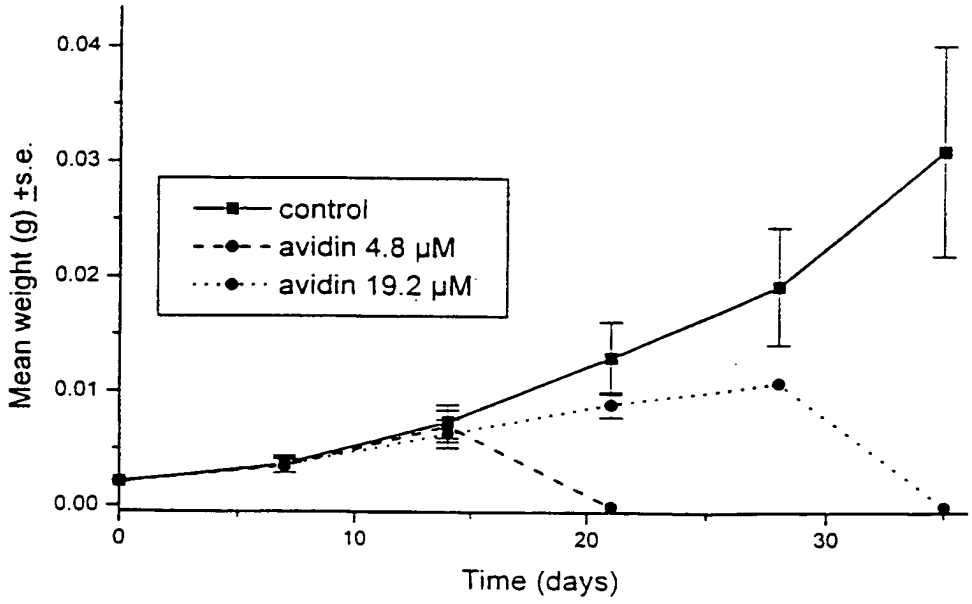


Figure 35

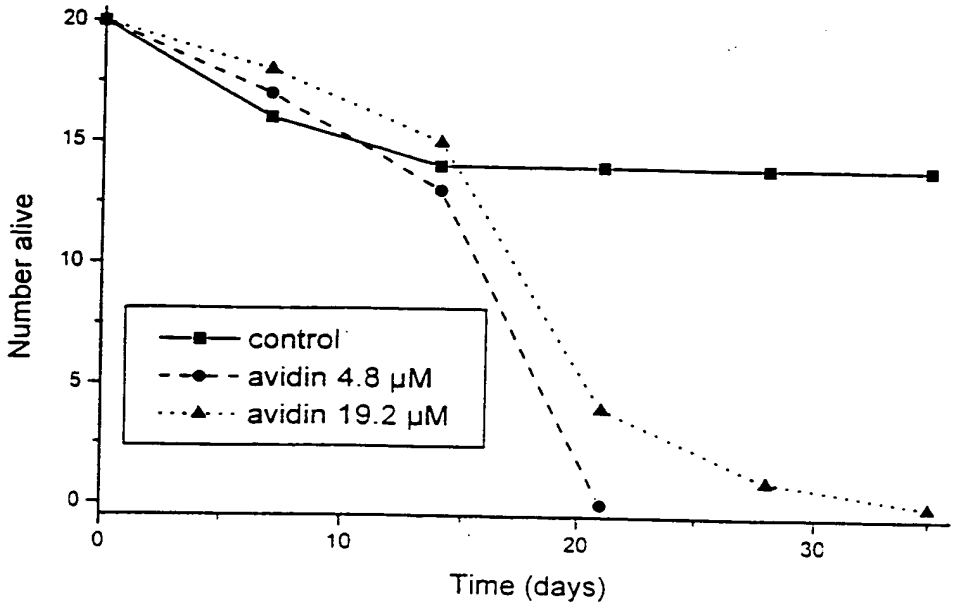


Figure 36

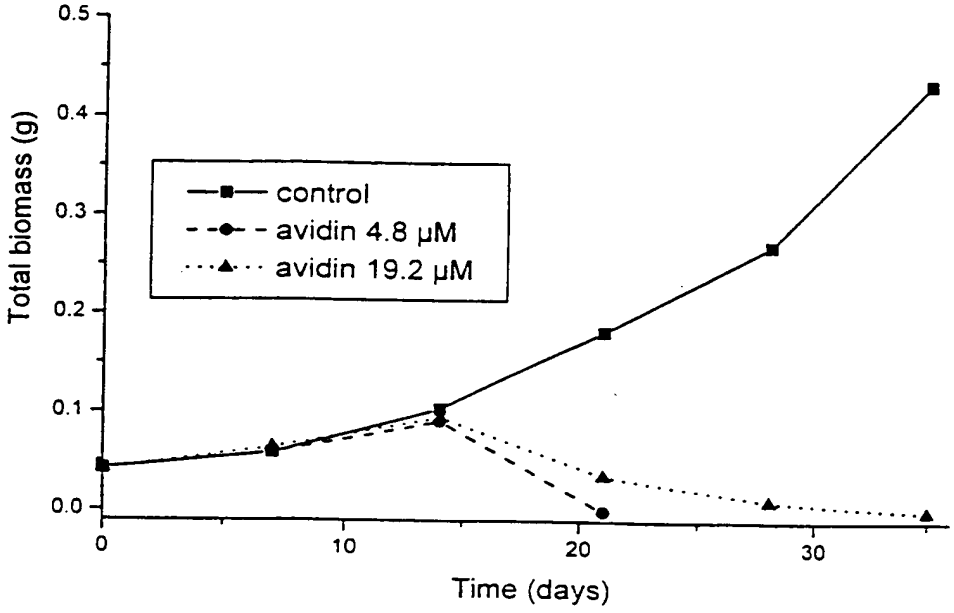


Figure 37

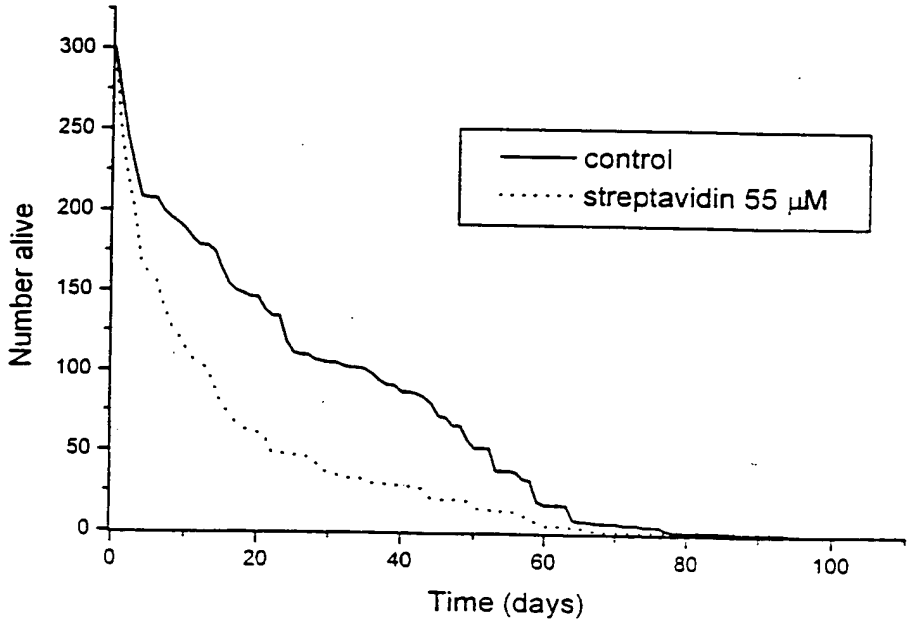


Figure 38

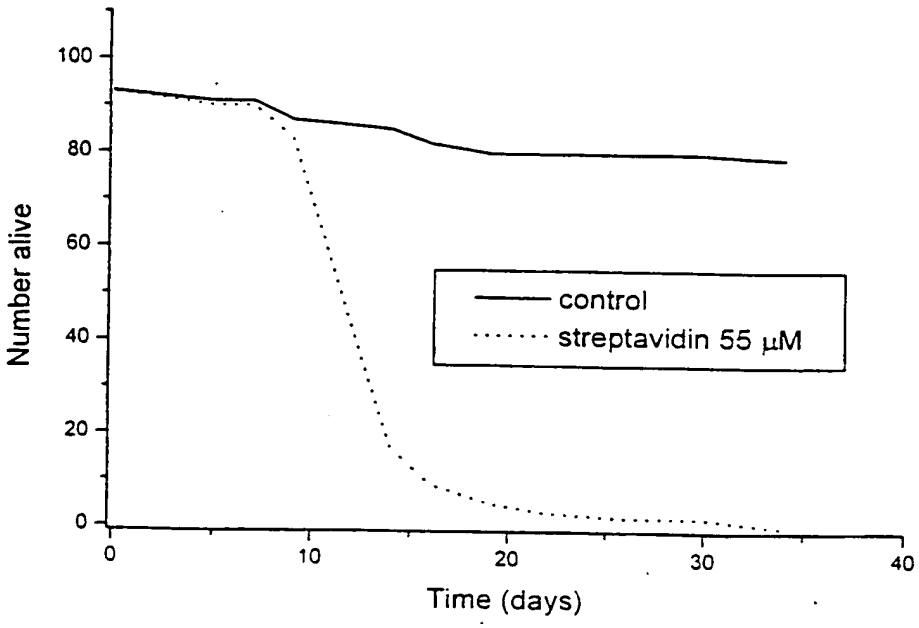


Figure 39

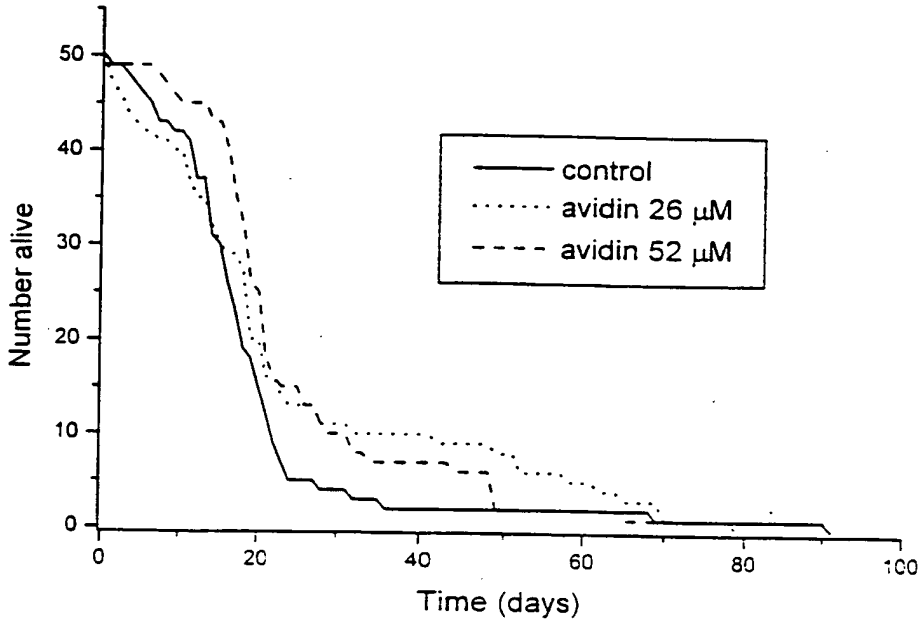


Figure 40

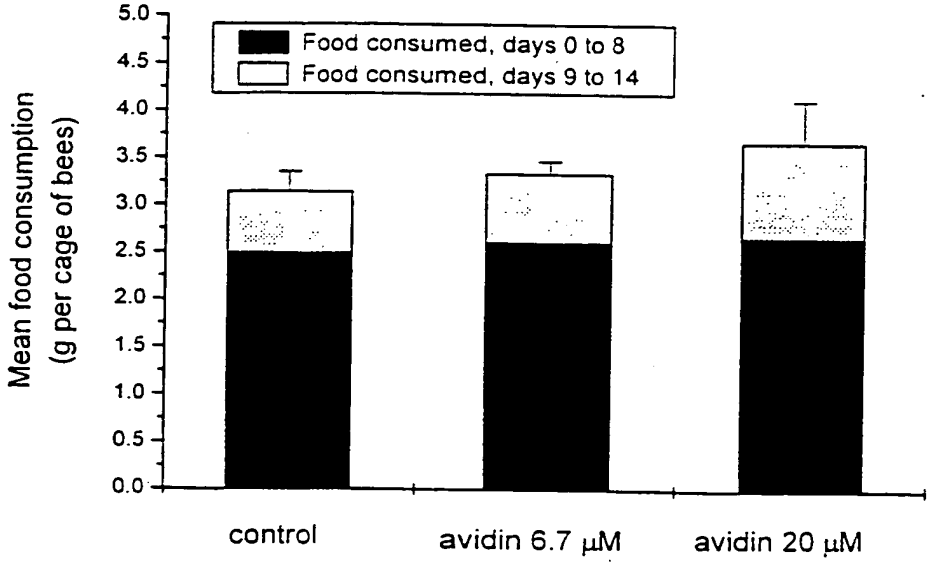


Figure 41

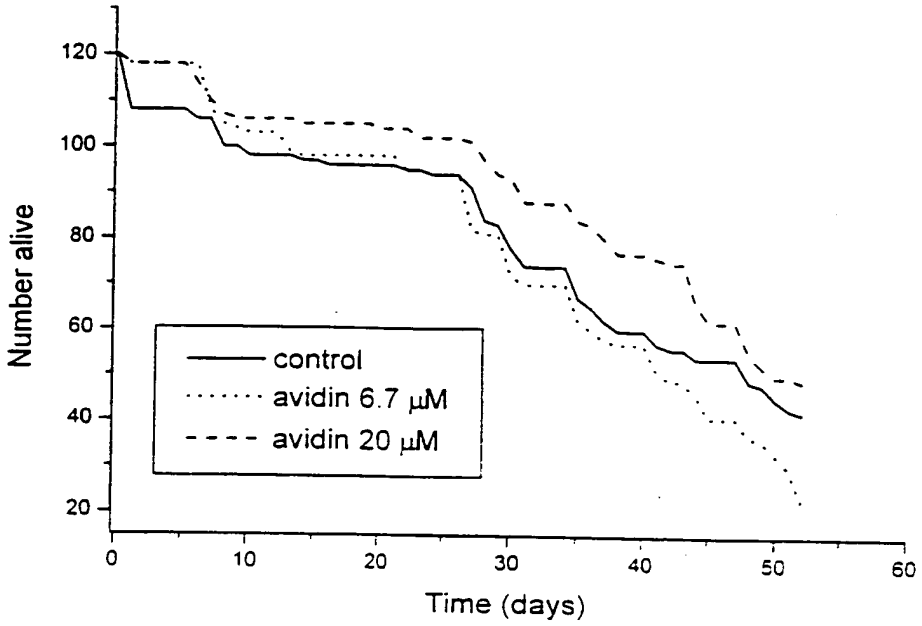


Figure 42

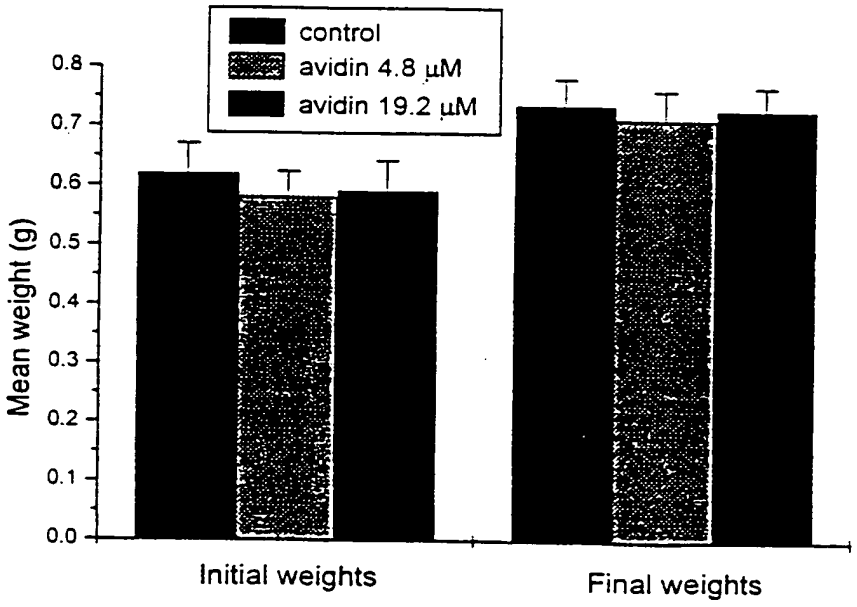


Figure 43

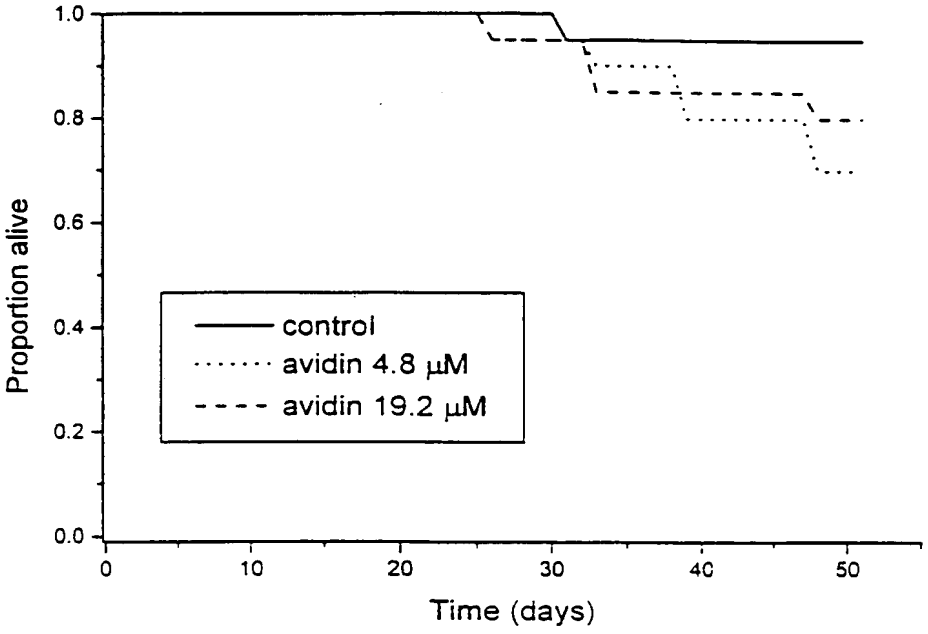


Figure 44

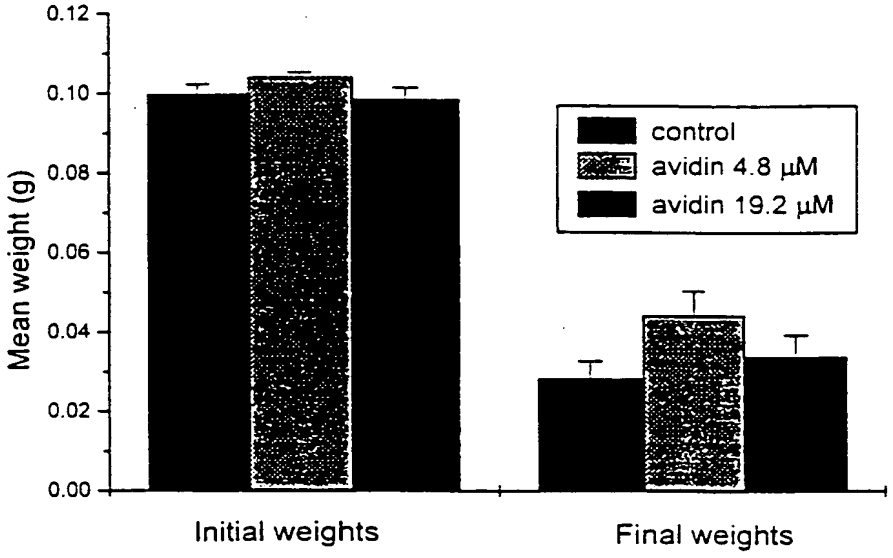


Figure 45

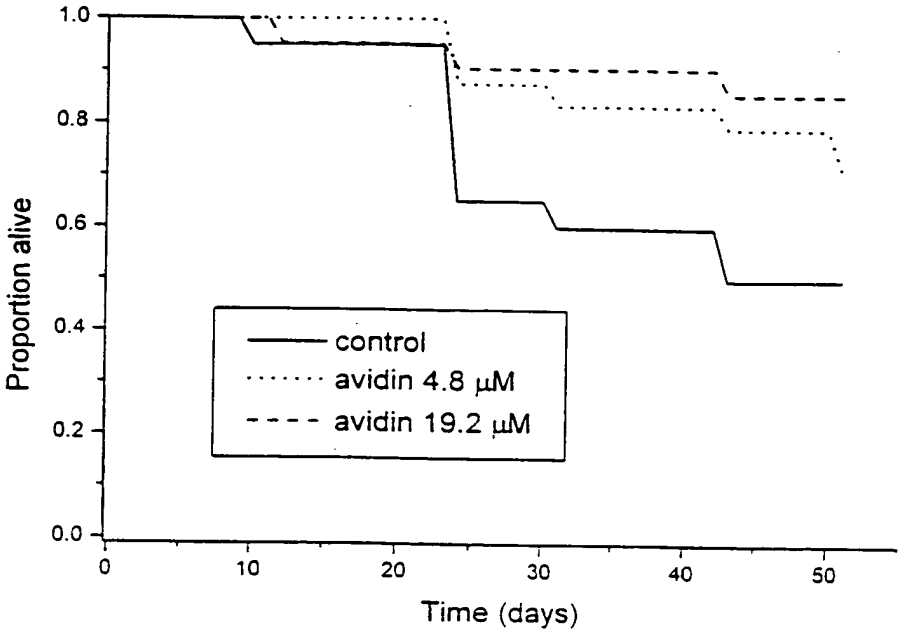


Figure 46

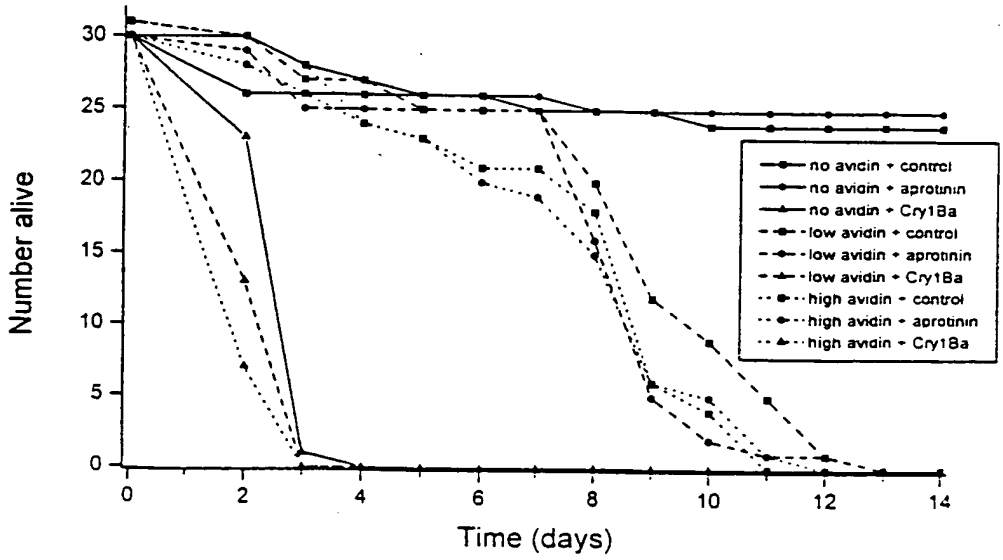


Figure 47

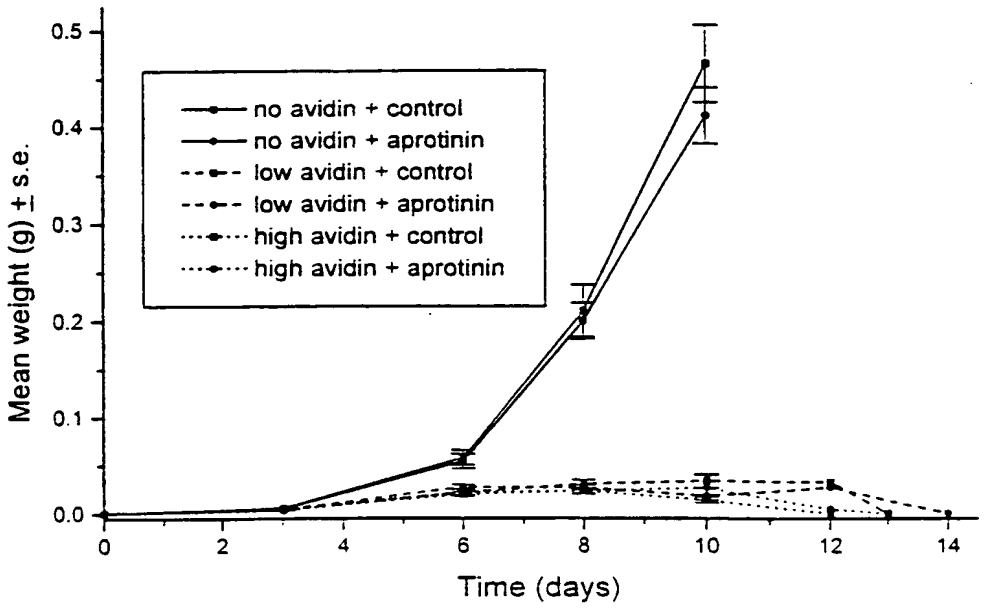


Figure 48

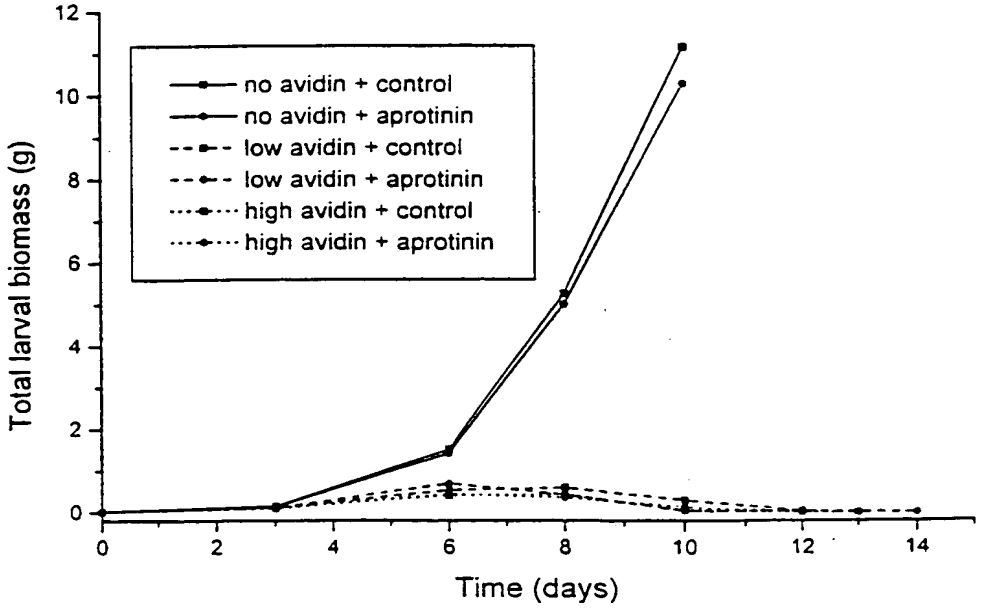


Figure 49